it is not functionally-available. Thus, Ogino does not teach each and every limitation of th present invention in a single prior art reference. Applicants respectfully submit that the rejections under 35 U.S.C. §102(b) be reconsidered and withdrawn.

With regard to all claims not specifically mentioned, these are believed to be allowable not only in view of their dependency on their respective base claims and any intervening claims, but also for the totality of features recited therein.

All claims are believed to be in condition for allowance. Should the Examiner disagree, Applicants respectfully invite the Examiner to contact the undersigned attorney for Applicants to arrange for a telephonic interview in an effort to expedite the prosecution of this matter.

CONCLUSION

In view of the foregoing amendments and accompanying remarks, reconsideration of the application and allowance of all claims are respectfully requested. A fee for a one-month extension of time is believed to be due for the amendments herein. Should any fee be required, please charge such fee to Procter & Gamble Deposit Account No. 16-2480.

Respectfully submitted,

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Appendix A

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Determination of Critical Micelle Concentration of Anionic Surfactants by Capillary Electrophoresis Using 2-Naphthalenemethanol as a Marker for Micelle Formation

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A method for the determination of the critical micelle concentration (CMC) of anionic surfactants by capillary electrophoresis (CE) has been developed. The method is based on the measurement of migration time of 2-naphthalenemethanol as a marker compound in CE, using varying concentrations of a desired surfactant in phosphate buffer (pH 7.0) as electrolyte solution. When the migration time of 2-naphthalenemethanol was plotted against the surfactant concentration, an inflection point appeared which corresponded to the CMC. By the CE method, the CMC values of seven anionic surfactants including sodium salts of alkyl sulfate, alkylsulfonic acid, fatty acid and bile acid were successfully determined; the CMC of sodium dodecyl sulfate determined by the CE method was 3.92 mM, which agreed closely with those measured by a dye solubilization method (3.53 mM) and a conductometric method (4.02 mM). The present CE method enabled the determination of CMC, if required, using as little as 300 µl or smaller volumes each of varying concentrations of surfactant solutions.

Keywords Critical micelle concentration, anionic surfactant, sodium dodecyl sulfate, capillary electrophoresis, micellar electrokinetic chromatography, 2-naphthalenemethanol

Micellar electrokinetic chromatography (MEKC)[1–3], a hybrid technique of capillary electrophoresis (CE) and partition chromatography, is a powerful tool for the separation of electrically neutral substances. In order to perform MEKC, a surfactant solution at the concentration higher than its critical micelle concentration (CMC) must be used as a separation solution. Therefore, knowing the CMC value of the surfactant is essential in MEKC experiments, especially when novel surfactants are used. A number of methods have been reported for the determination of CMC, such as surface tension[4], electric conductivity[5] and dye solubilization[6] procedures. However, these methods are tedious and time-consuming and/or require large volumes of surfactant solutions. In this study, we devised a method for the determination of the CMC of anionic surfactants by CE, based on the abrupt changes in the migration time of a suitable electrically neutral marker compound using varying concentrations of the desired surfactant solutions as migration solutions. At below CMC, the marker is thought theoretically to migrate on the electro-osmotic flow generated by the dissociation of silanol groups on the inner wall of the fused-silica capillary. However, at above CMC, the migration time of the marker should increase with increasing surfactant concentration, because the neutral compound begins to be incorporated into the micelles. Actually, the plot of migration time and surfactant concentration gave two straight lines whose intersection corresponds to a CMC value.

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Experimental

Chemicals

Sodium decyl sulfate, sodium tetradecyl sulfate, 2-naphthol, toluene, sodium dihydrogenphosphate and disodium hydrogenphosphate were purchased from Kanto Chemical (Tokyo, Japan). Sodium dodecyl sulfate (SDS), sodium decanesulfonate, sodium cholate, sodium deoxycholate and benzylalcohol were obtained from Nacalai Tesque (Kyoto, Japan). Sodium laurate and Sudan III were purchased from Tokyo Kasei (Tokyo, Japan) and 2-naphthalenemethanol was from Aldrich (Milwaukee, WI, USA). All chemicals used were of analytical-reagent grade. Water used was purified on a Milli RO-Milli Q system (Millipore, Bedford, MA, USA).

For the determination of CMC by CE, dye solubilization and electric conductivity methods, various concentrations of anionic surfactants (up to 1.5 – 2 times those of CMC reported in literature[7,8]) were prepared in 20 mM sodium phosphate buffer (pH 7.0), except for sodium tetradecyl sulfate, which was dissolved in 5 mM sodium phosphate buffer (pH 7.0). Compounds tested as markers in CMC measurements were as follows:

benzylalcohol (used as a 10 mM aqueous solution), toluene (used as toluene itself), Sudan III (used as a saturated solution in methanol), 2-naphthol (used as a 5 mM aqueous solution) and 2-naphthalenemethanol (used as a 1 mM aqueous solution).

Conditions for determination of CMC by CE

The CE experiments were performed with a JASCO CE-990 system (JASCO, Tokyo, Japan). Unmodified fused-silica capillaries (50 μm i.d.×70 cm; 50 cm from the inlet to the detector) were used throughout. The CE-990 system was operated under following conditions: injection, pressurized method (20 mbar for 6 s) from the anodic end; applied voltage, 20 kV; temperature, 40°C; wavelength, 210 nm (toluene, benzylalcohol, Sudan III and 2-naphthol) and 275 nm (2-naphthalenemethanol). Usually, CE was performed using a 4-ml anodic buffer reservoir and a 25-ml cathodic buffer reservoir, both filled with the surfactant solutions (method I). For the microdetermination of CMC, only the anodic buffer reservoir was filled with the surfactant solution and the 25-ml cathodic reservoir was filled with the phosphate buffer alone. In this case, a 4-ml reservoir (method II) or a 300-μl microvial attachment (method III) was used as the anodic reservoir.

Dye solubilization method

Sudan III (5 mg) was added to 5 ml of the varying concentrations of surfactant solutions and the mixtures were saturated by shaking overnight at 40°C. Then, the excess Sudan III was filtered off by passing through a membrane filter (0.45 μm, DISMIC-25CS, Advantec Toyo, Tokyo, Japan). Absorbance of the solution was measured at 505 nm with a Hitachi 100-40 spectrophotometer (Hitachi, Tokyo, Japan).

Conductometric method

The conductivity of the varying concentrations of surfactant solutions was measured with a TOA Electronics CM-40s conductometer (TOA Electronics, Tokyo, Japan) at 40°C.

Results and Discussion

Selection of marker compound

Initially, we searched for a marker compound suitable for the determination of the CMC of anionic surfactants. The marker should satisfy the following properties: (1) high UV absorptivity to make its detection easy, (2) hydrophobicity (electrical neutrality) to be incorporated into micelles and (3) proper solubility in water to be dissolved in buffer. Sudan III, toluene, benzylalcohol, 2-naphthol and 2-naphthalenemethanol were tested using varying concentrations of SDS as migration solutions for CE. Sudan III was not usable owing to its poor solubility in water. Benzylalcohol was hardly incorporated into SDS micelles. Toluene was found to be incorporated into SDS micelle, however, no inflection point appeared in the plot of the migration time of toluene and SDS concentration (Fig. 1). On the contrary, 2-naphthol and 2-naphthalenemethanol were found to be suitable as the marker compounds in preliminary experiments, giving clear inflection points by which the CMC values (about 4 mM) were easily read off. Finally, 2-naphthalenemethanol was selected because it has no dissociative functional group and afforded more clear inflection points in many cases.

Determination of CMC of various anionic surfactants

CMC of five anionic surfactants were determined by the CE method I. To check the reliability of the recommended CE method, the dye solubilization and the conductometric methods described in experimental section were also carried out. Figure 2 shows plots of each physicochemical parameter against surfactant concentrations. Except for sodium cholate (Fig. 2e), which did not show a clear inflection point by the conductometric method, two straight lines observed on these plots were extrapolated to find the point of intersection using the least-squares method. The CMC calculated from the plots are summarized in Table 1. The results obtained by the CE method agreed well with those obtained by the dye solubilization method and the conductometric method. Reproducibility of the CE method was satisfactory; when the determination of CMC of SDS was repeated five times, the CMC was calculated to be 3.88±0.15 mM (mean±standard deviation) and the relative standard deviation was 3.9%.

The CMC of other typical anionic surfactants such as sodium laurate and sodium deoxycholate were also measured by the CE method. The CMC values determined were 7.15 mM and 4.16 mM, respectively. However, their CMC could not be determined by the dye solubilization method. The reason is unknown.

The CMC values observed here were considerably lower than the literature values.[7,8] However, this fact is

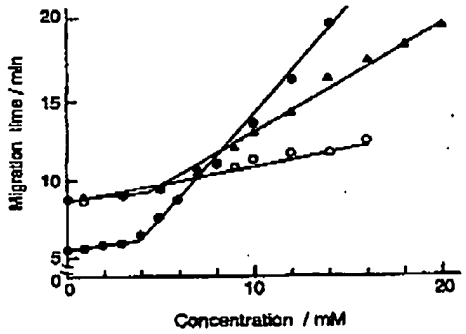

Fig. 1 Effect of marker compounds on determination of CMC of SDS by CE. Marker: ○, toluene; ●, 2-naphthalenemethanol; ▲, 2-naphthol.

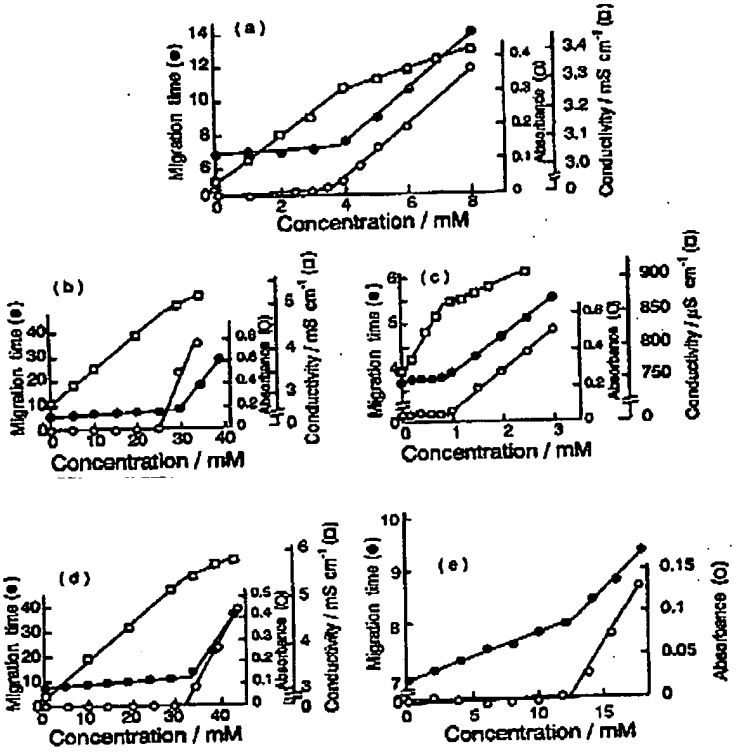

Fig. 2 Determination of CMC of (a) SDS, (b) sodium decyl sulfate, (c) sodium tetradecy sulfate, (d) sodium decanesulfonate and (e) sodium cholate by CE (●), dye solubilization (○) and conductometric (□) methods.

not surprising because the CMC measurements were done in the presence of salts as the buffer component, which are known to lower the CMC.[9]

Microdetermination of CMC by CE method II and III

Minimizing the consumption of surfactant required for the CMC measurement was attempted on the basis of the following consideration. In principle, the anionic surfactants are only required in the anodic vessel but not in the cathodic one, since 2-naphthalenemethanol injected from the anodic end migrates toward the cathodic end together with the surfactant. To demonstrate the idea, the CMC of five surfactants was determined by CE using a 4-ml anodic vial filled with surfactant solutions and a 25-ml cathodic vial filled with the phosphate buffer (method II). The results are summarized in Table 1. Furthermore, the volume of surfactant solution required for the determination of CMC can be reduced to only 300 μl or lower volumes using a microvial attachment as the anodic reservoir (method III). Under the condition, CMC of SDS was calculated

Table 1 CMC values of some anionic surfactants determined by CE, dye solubilization and conductometric methods

Surfactant	CMC[a]/mM		
	CE[b]	Dye solubilization	Conductometry
SDS	3.92 (3.69)	3.53	4.02
Sodium decyl sulfate	30.3 (27.0)	25.0	26.9
Sodium tetradecyl sulfate	0.87 (0.71)	0.86	0.89
Sodium decanesulfonate	34.5 (30.4)	32.9	34.3
Sodium cholate	12.8 (13.5)	12.6	—[c]

a. Values are means of two determinations.
b. Determined by the method I (using a 4-ml anodic reservoir and a 25-ml cathodic reservoir, both filled with surfactant solutions). The values in parentheses are the CMC obtained by method II (using a 4-ml anodic reservoir filled with surfactant solutions and a 25-ml cathodic reservoir filled with the phosphate buffer).
c. Not determined.

to be 3.50 mM. Thus the CMC was found to be also determined by the micro CE methods in good agreement with those (3.92 mM) obtained by the normal operation.

The CE method described here is easy to perform with small volumes of surfactant solutions and is applicable to various types of anionic surfactants including sodium salts of alkyl sulfate, alkylsulfonic acid, fatty acid and bile acid, some of which could not be determined by dye solubilization and conductometric methods. Recently, Jacquier and Desbène[10] have reported a method for the determination of the CMC of surfactants by CE. Their concept is essentially the same as ours. However, the use of naphthalene as the marker makes practical use difficult due to its low solubility in water; thus its application is limited only to SDS. In conclusion, our method seems to be useful for the microdetermination of the CMC of anionic surfactants because the method possesses several advantages over conventional methods.

A part of this work has been reported in the graduation thesis of K. Matsuura (Science University of Tokyo, Tokyo, 1995).

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